SESSION 4: PRACTICAL APPROACH/ HUMAN PERSPECTIVE

007 Prevalence of coagulase negative staphylococci on dairy farms in The Netherlands

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Introduction: Coagulase-negative staphylococci (CNS) are considered to be minor mastitis pathogens. However, CNS are isolated from cases of subclinical and clinical mastitis cases, and also from teat canals, teat skin and teat ducts (1,2). Additionally CNS are the predominant pathogens in heifer mastitis (4,5). The aim of this study was to analyse the prevalence of intramammary infection (IMI) with CNS species in Dutch dairy cows and to study possible risk factors associated with prevalence of CNS IMI.

Materials and Methods: In this survey, 49 herds were randomly selected stratified per province. Herds were included that had at least 40 lactating cows and participated in the Dutch milk recording system. Milk samples were collected from all quarters of a selection of cows, based on cow somatic cell count (SCC) of the last milk recording before the farm visit. Two groups of cows were selected: 1) a high SCC (HSCC) group that consisted of all cows and heifers with a SCC >250,000 cells/ml and >150,000 cells/ml, respectively, and 2) a low SCC (LSCC) group that consisted of approximately 25% of cows and heifers with a SCC ≤250,000 cells/ml and ≤150,000 cells/ml, respectively. Quarter foremilk samples were collected aseptically by personnel of the Animal Health Service (AHS). Bacteriological culturing was performed according to the NMC protocols (3).
A selection of CNS was tested with the API Test, (BioMerieux). The Apilab software was used to calculate the probability of the identification result in a range of 10 to 100%. Possible tests were suggested when needed for the delineation of species.
A questionnaire was conducted to obtain management information of the farms such as housing facilities, milking procedures, feeding and treatment regimes. The association between these management factors and prevalence of CNS IMI was determined using a generalized linear model. All variables with a *P*-value < 0.25 in a univariable analysis were offered to the multivariable analysis. The final model was constructed by means of a backwards elimination method, checking for possible confounders and interactions. Variables with *P*-value > 0.05 (T-test) were removed from the model. All possible two-way interactions between remaining significant variables were investigated. Normality of the data was checked on the residuals and the fit of the model was given by the adjusted R^2.

Results: Bacterial growth occurred in 37.7% of the 2174 collected milk samples from HSCC cows and in 21.1% of the 2046 collected milk samples from LSCC cows ($P<0.0001$). Coagulase-negative staphylococci were the most frequently isolated group of bacteria in both the HSCC and the LSCC group, 14.6% and 10.4% respectively. The difference in prevalence of CNS IMI between these two groups was significant ($P<0.05$). The prevalence of mastitis pathogens differed among herds. Coagulase-negative staphylococci were found in quarter milk samples of all farms. Distribution of CNS differed considerably among parity groups. 49.4% of the heifers had one or more quarters shedding CNS, this was 30.9 and 33.1% in second and third parity cows, respectively (Figure 1). From the 530 isolated CNS, 158 were offered for identification with the API test. In total 14 species were identified: the predominant CNS species was *Staph. chromogenes* (30.3%) followed by *Staph. epidermidis* (12.9%) and *Staph. simulans* (11.0%). *Staphylococcus haemolyticus* was significantly more prevalent in

the LSCC group in comparison with the HSCC group. The prevalence of the identified species did not differ between the parity groups.
Quarters with a CNS IMI had a geometric mean quarter SCC of 109,000 cells/ml, which was almost twice as high of the culture-negative quarters which had a quarter SCC of 58,000 cells/ml ($P<0.05$). *Staphylococcus epidermidis* and *Staph. simulans* had significant higher SCC in the HSCC in comparison with the LSCC group ($P<0.05$). The SCC of the other species did not differ between the two groups.
After the univariable analysis of the questionnaire 29 variables remained. In the final model only three variables remained, but the model showed a good fit to the data (adjusted R^2 0.89). Factors that were associated with an increased prevalence of CNS IMI were using ditchwater instead of tap water as drinking water ($P=0.02$), pasturing the cows during summer instead of keeping the cows indoors ($P=0.008$) and using one instead of two dry cow groups of ($P=0.03$). In the final model production level, post-milking teat disinfection, dry cow therapy, housing facilities around calving and type of bedding did not attribute to the difference in the prevalence of CNS IMI between the herds studied.

Conclusion: In this study, the prevalence of CNS in The Netherlands was estimated on 49 farms with at least 40 lactating cows. The farms were randomly selected from the national database. In total, 4220 quarter milk samples were collected; the predominant subclinical mastitis pathogens were CNS. Fourteen species of CNS were identified; the predominant species was *Staph. chromogenes* (30.3%) followed by *Staph. epidermidis* (12.9%) and *Staph. simulans* (11.0%). The prevalence of CNS was higher in heifers in comparison with older cows. Geometric mean quarter SCC of CNS-positive quarters was 109,000 cells/ml, which twice as high in compared to culture-negative quarters (58,000 cells/ml). The multivariable analysis on the questionnaire of management information showed that using ditchwater, pasturing during the summer and using one instead of two groups of dry cows increased the prevalence of CNS IMI.

References:

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Figure 1: Parity distribution of intramammary infection with coagulase negative staphylococci.